AMENDMENTS TO THE DRAWINGS

The attached sheets of drawings include changes to Figures 1-5. These sheets of Figures 1-5 replace the previously submitted sheets of Figures 1-5. In Figures 1-5, previously added designations (A, B, C, etc.) for each individual structure have been deleted in compliance with 37 C.F.R. 1.121(d). No new matter has been added.

Attachments: Replacement Sheets

REMARKS

Amendments to the Claims

Claims 23, 41 and 49 have been amended to revise "tumors associated with angiogenesis" to "tumors sensitive to thalidomide" solely to promote the allowance of the case and without acquiescing to the Examiner's rejection. The claims have been amended to recite methods for inhibiting growth, metastasis or recurrence of tumors sensitive to thalidomide, by administering an effective amount of thalidomide to human patients. The claims are amended to more accurately define the subject matter of the present claims. The claims are supported by the originally filed specification and no new matter has been introduced by the amendments. Their entry is respectfully requested. Upon entry of the present amendments, claims 23, 25-31 and 33-71 are pending in this application.

I. The Objection to the Drawings Should be Withdrawn

On page 2 of the Office Action, the drawings are objected to because they include the added designations of structures (A, B, C, etc.). In response to the objection, Applicant has amended the drawings by deleting the designations as proposed by the Examiner. No new matter has been added. Accordingly, Applicant respectfully requests that the objection to the drawings be withdrawn.

II. The Claimed Invention Meets Enablement Requirements

Claims 23, 25-31 and 33-71 are rejected under 35 U.S.C. §112 as failing to comply with the enablement requirement. (pages 3-12 of Office Action). Applicant respectfully traverses the rejection.

The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation. *U.S. v. Telectronics, Inc.*, 857 F.2d 778, 785 (Fed. Cir. 1988). As to the meaning of undue experimentation, the Office Action states that "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a <u>reasonable amount of guidance</u> with respect to the direction in which experimentation should proceed to enable the determination of how to practice a desired embodiment of the claimed invention." (Pages 5-6 of the Office Action). (emphasis added). Applicant respectfully submits that the pending claims comply with the enablement requirement, because the specification provides a reasonable amount of guidance to practice the claimed invention, as discussed below.

The pending claims encompass methods for inhibiting formation or growth of tumors, inhibiting metastasis of tumors, and reducing the recurrence of tumors in humans, using an effective amount of thalidomide.

The Examiner admitted that the specification discloses formulations to be used (page 20, lines 22-24), and mode of administration and doses of thalidomide (page 21, lines 1-10). (Page 4 of the Office Action). Indeed, page 20, line 22 to page 23, line 23 of the specification discloses the dosage forms, formulations, routes of administration and doses of thalidomide to be administered.

Nonetheless, the Office Action states that the formulations and administration routes taught in the specification are traditionally used for all therapeutic agents, regardless of the condition to be treated or the agent being administered, and that the specification provides no specific guidance over that which is found throughout the prior art. (Page 4 of the Office Action).

It is respectfully submitted that Examiner's comment is not relevant to the enablement standards. Whether the formulations and administration routes disclosed in the instant specification are or can be used for other agents, is not relevant to the assessment of the enablement of the claim. The test of enablement is whether those skilled in the art would have been able to make and use the claimed invention based on the disclosure of the specification. *See U.S. v. Telectronics, Inc.*, at 785. Applicant respectfully submits that the pending claims are enabled, because the specification contains a teaching of the manner and process of making and using an invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented. *Id.*

Specifically, the specification discloses the methods of administering thalidomide to patients having tumors, including mode of administration (*e.g.*, page 20, lines 22-34 and page 21, lines 11-23 for claims 25, 43 and 61), doses of thalidomide (*e.g.*, page 21, lines 1-10 for claims 27-29, 36-38 and 53-55), and dosage forms and formulations (*e.g.*, page 21, line 24 to page 23, line 23 for claims 26, 31, 39, 40, 48, 57 and 60).

Thus, one skilled in the art would have been able to practice the claimed invention by administering the specified amount of thalidomide using the specified routes of administration to the specified patients, in accordance with the explicit teachings of the present application.

The specification also discloses working Examples I to III (pages 24-30, chick chorioallantoic membrane (CAM) assay, rabbit cornea angiogenesis assay and inhibition of bFGF induced corneal neovascularization), demonstrating that thalidomide is effective in

inhibiting angiogenesis *in vivo*. The inhibitions of angiogenesis by thalidomide are described on page 30 of the specification, and Figures 6 and 7.

The Office Action has recognized the correlation between tumor growth and angiogenesis, and has not questioned the relationship. (Pages 3 and 5 of the Office Action).

Applicant respectfully points out that "[a]s long as the specification discloses at least one method for making and using the claimed invention that bears a reasonable correlation to the entire scope of the claim, then the enablement requirement of 35 U.S.C. § 112 is satisfied. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18 (CCPA 1970) and MPEP § 2164.01(b).

Thus, from the description of the specification and the knowledge in the art, one skilled in the art would have been able to appreciate that the inhibition of angiogenesis by administration of thalidomide would lead to the treatment or inhibition of tumors. The enablement requirement is met if the description enables any mode of making and using the claimed invention. *CFMT, Inc. and CFM Technologies, Inc. v. Yieldup International Corp.*, 349 F.3d 1333, 1338 (Fed. Cir. 2003).

In view of the foregoing, the specification provides a sufficient guidance as to inhibiting growth, metastasis or recurrence of tumors associated with angiogenesis, by administering an effective amount of thalidomide.

Nevertheless, the Office Action states that the prior art as a whole supports that thalidomide is not safe and effective treatment for tumors, and that the working examples are limited to demonstrating the anti-angiogenic activity of thalidomide in animal models. (Pages 4-11 of the Office Action).

Applicant respectfully points out that for purposes of the enablement requirement under 35 U.S.C. § 112, "[t]he applicant need not demonstrate that the invention is completely safe." MPEP § 2164.01(c) (emphasis added). Further, it is respectfully submitted that the enablement requirement does not mean that disclosure has to enable one of ordinary skilled in the art to make and use perfected and commercially viable embodiment. *CFMT, Inc. and CFM Technologies, Inc. v. Yieldup International Corp.*, 349 F.3d 1333, 1338 (Fed. Cir. 2003).

Applicant respectfully submits that the Examiner may be confusing the requirements under law for obtaining a patent with the requirements for obtaining government approval for marketing a particular drug for human consumption. For example, in *In re Brana*, the PTO alleged that animal testing was not reasonably predictive of the success of the claimed compounds for treating cancer in humans. 51 F.2d 1560, 1567, 34 U.S.P.Q.2d 1436 (Fed Cir. 1995). The Court rejected this argument and stated that "[t]he Commissioner, as did the Board, confuses the requirements under the law for obtaining a patent with the requirements

for obtaining government approval to market a particular drug for human consumption." *Id.* The Court further stated that "Title 35 does not demand that such human testing occur within the confines of the Patent and Trademark Office Proceedings." *Id.*, citing *Scott v. Finney*, 34 F.3d 1058, 1063, 32 U.S.P.Q.2d (BNA) 115, 120 (Fed. Cir. 1994) (emphasis added). Thus, Applicant respectfully submits that Applicant is not required to provide any human data or the safety of the claimed invention.

Next, the Office Action states the fact that thalidomide inhibits angiogenesis does not reasonably suggest that it will be effective in inhibiting tumor growth. (Page 11 of the Office Action). Applicant respectfully disagrees.

The specification clearly describes the relationship between the inhibition of angiogenesis and the inhibition of tumor growth. For example, the specification at page 2, lines 23-30; page 4, lines 27-34 and page 5, line 9 to page 6, line 12, states as follows:

> "Persistent, unregulated angiogenesis occurs in a multiplicity of disease states, tumor metastasis and abnormal growth by endothelial cells and supports the pathological damage seen in these conditions. The diverse pathological states created due to unregulated angiogenesis have been grouped together as angiogenic dependent or angiogenic associated diseases. Therapies directed at control of the angiogenic processes could lead to the abrogation or mitigation of these diseases....
>
> One of the most frequent angiogenic diseases of childhood is the hemangioma.....
>
> Angiogenesis is prominent in solid tumor formation and metastasis. Angiogenic factors have been found associated with several solid tumors such as rhabdomyosarcomas, retinoblastoma, Ewing sarcoma, neuroblastoma, and osteosarcoma. A tumor cannot expand without a blood supply to provide nutrients and remove cellular wastes. Tumors in which angiogenesis is important include solid tumors, and benign tumors such as acoustic neuroma, neurofibroma, trachoma and pyogenic granulomas. Prevention of angiogenesis could halt the growth of these tumors and the resultant damage to the animal due to the presence of the tumor.
>
> It should be noted that angiogenesis has been associated with blood-born tumors such as leukemias. It is believed that angiogenesis plays a role in the abnormalities in the bone marrow that give rise to leukemia-like tumors.
>
> Angiogenesis is important in two stages of tumor metastasis. The first stage where angiogenesis stimulation is important is in the vascularization of the tumor which allows tumor cells to enter the blood stream and to circulate throughout the

body. After the tumor cells have left the primary site, and have settled into the secondary, metastasis site, angiogenesis must occur before the new tumor can grow and expand. Therefore, prevention of angiogenesis could lead to the prevention of metastasis of tumors and possibly contain the neoplastic growth at the primary site.

Knowledge of the role of angiogenesis in the maintenance and metastasis of tumors has led to a prognostic indicator for breast cancer....Control of angiogenesis by therapeutic means could possibly lead to cessation of the recurrence of the tumors."

Thus, the specification clearly describes that the inhibition of angiogenesis with thalidomide would lead to the inhibition of tumor growth or tumor metastasis.

In addition, in the specification at pages 24-30 of the present application, Examples I to III demonstrate that thalidomide is effective in inhibiting angiogenesis *in vivo*. The prior art at the time of filing date reports that the CAM assay and rabbit cornea assay described in the present application are known in the art to be commonly-used assays for studying anti-angiogenic activity *in vivo*; that the recited tumors (solid tumors, retinoblastoma and hemangioma) are angiogenesis-dependent diseases; and that angiogenesis inhibitors can be used in the treatment of these diseases. *See e.g.* previously submitted Langer *et al.*, *Biotechnology (NY)*, 9(7) (1991), page 630. Thus, the specification and prior art supports the use of the assay described in the specification as an indicator of activity against angiogenesis and tumors. *See In re Brana* at 1566.

Where a particular model is recognized as correlating to a specific condition in a given art, the Examiner should accept that correlation, unless the Examiner has evidence that the model does not correlate. *In re Brana* at 1566; MPEP. § 2164.02. The Office Action has not provided with any evidence that the animal model disclosed in the specification does not correlate with the inhibition of tumors.

In the last response, Applicant also submitted the articles which reported that thalidomide had been being studied and used for treating tumors, based on the discovery of the present inventor that thalidomide inhibits angiogenesis. The article of the present inventor D'Amato study (*Proc. Natl. Acad. Sci.*, USA 91, 1994, page 4082-5) also describes that thalidomide was effective in inhibiting angiogenesis *in vivo* in rabbit cornea angiogenesis assay, and concludes that there are clear implications for the use of this drug for treating angiogenesis associated diseases including tumors. *See*, at page 4085.

Further, Applicant respectfully points out that the article submitted herewith teaches that angiogenesis is important in tumor progression, that thalidomide can inhibit angiogenesis in animal models, and that angiogenesis-inhibiting drug thalidomide may be useful for

treating cancers. *See*, Singhal *et al.*, Antitumor Activity of Thalidomide in Refractory Multiple Myeloma, *N. Engl. J. Med.*, 1999, page 1565, 2nd paragraph of right column. Indeed, the author referred to the present inventor's animal model studies of angiogenesis, which are described in the instant specification. *Id.* at 1565 and 1571.

Therefore, the art as a whole supports the use of thalidomide for inhibiting tumors, based on the discovery of the present inventor that thalidomide inhibits angiogenesis. This is evidence that a skilled person in the art can practice the claimed invention without undue experimentation based upon the examples and the specification.

Nonetheless, the Office Action states that the articles cited by the Examiner disclose ineffectiveness of thalidomide against cancer, and thus they support lack of enablement of the claimed invention. (Office Action, pages 7-10). The Office Action also states that Applicant's previous arguments on obviousness rejections that the cited references do not disclose effectiveness of thalidomide against cancer, provide evidence for lack of enablement. (Office Action, page 10). The test of enablement is whether a sufficient guidance is provided in the specification so as to allow those of ordinary skill in the art to make and use the claimed invention. The cited references and arguments are not relevant to the assessments of enablement, because the specification explicitly describes the methods of administering the specified amount of thalidomide for the specified routes of administration to the specified patients. "[i]f a statement of utility in the specification contains ... a connotation of how to use, and/or the art recognizes that standard modes of administration are known and contemplated," the enablement requirement is satisfied. *In re Brana*, at 1566; MPEP § 2164.01(c). Further, in the last response, Applicant submitted several articles which report on the inhibition of tumors and angiogenesis by thalidomide, evidencing that one in the art can practice the claimed invention from the specification without undue experimentation. The Office Action has not provided any evidence contradicting the studies of the articles submitted by Applicant.

Further, it is alleged that although many conditions related to angiogenesis are contemplated to be treatable with thalidomide, Applicant has not provided any guidance on how one would specifically treat any particular disease. (Page 11 of the Office Action). Applicant respectfully disagrees.

Applicant respectfully submits that even in unpredictable arts, a disclosure of every operable species is not required to satisfy enablement. Compliance with the enablement requirement does not turn on whether an example is disclosed. *Gould v. Quigg*, 822 F.2d 1074, 1078 (Fed. Cir. 1987). The law makes clear that the specification need teach only one mode of making and using a claimed invention. *Amgen Inc. v. Hoechst Marion Roussel, Inc.*,

314 F.3d 1313, 1335 (Fed. Cir. 2003). The specification need not explicitly teach those in the art to make and use the invention; the requirement is satisfied if the specification teaches those in the art enough that they can make and use the invention without undue experimentation. *Id.*, at 1334. One of ordinary skill in the art, armed with the information presented in the specification and publication, has adequate guidance to practice the claimed invention.

Applicant respectfully submits that an *in vitro* or *in vivo* animal model examples in the specification, in effect, constitute working examples, as the examples correlate with a claimed method invention. *In re Brana*, at 1566; MPEP § 2164.02. "A rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence." *See Cross v. Iizuka*, 753 F.2d 1040, 1050 (Fed. Cir. 1985)

With this legal framework in mind, Applicant submitted publications to show the correlations between the claimed methods and the animal models for angiogenesis inhibition described in the specification. *See e.g.* Langer *et al.*, at 630, right column. Applicant notes that human working examples are not required under 35 U.S.C., first paragraph.

In view of the foregoing, it is clear that a sufficient guidance is provided in the specification so as to allow those of ordinary skill in the art to make and use the claimed invention.

Further, Applicant notes that some factors that may --but need not[1]-- be considered in determining whether experimentation is undue include the quantity of experimentation necessary and the amount of direction or guidance provided. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). In *In re Wands*, the Court of Appeals for the Federal Circuit held that claims directed to immunoassay methods <u>were</u> enabled even though in order to practice the claimed invention, one would have to screen "hybridomas to determine which ones secrete antibody with desired characteristics." This was because "[p]ractitioners of this art are prepared to screen negative hybridomas in order to find one that makes the desired antibody." *Id.* at 740.

The claimed invention is directed to the use of obtainable compound, for which routes of administration and amounts are set forth in the specification on pages 20-23. The determination by a physician as to whether thalidomide is effective in treating the recited disease in a given patient is a type of determination that is always made by physicians for every pharmaceutical. Indeed, the determination is a routine one that every physician is

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[1] *Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 927 F.2d 1200, 1230 (Fed. Cir. 1991), *cert. denied*, 502 U.S. 856 (1991) ("it is not necessary that a court review all the *Wands* factors to find a disclosure enabling. They are illustrative, not mandatory.").

prepared to make, and which requires little or no effort. Therefore, Applicant respectfully submits that one reasonably skilled in the art could make or use the invention as claimed without undue experimentation.

In sum, Applicant respectfully submits that the specification provides sufficient information and guidance to those of ordinary skill in the art to make and use the claimed invention, and that to the extent any experimentation is necessary, such experimentation is not undue. Therefore, Applicant respectfully requests that the rejection of the claims under 35 U.S.C. § 112, first paragraph be reconsidered and withdrawn.

III. <u>The Double Patenting Rejection Should Be Withdrawn</u>

Claims 23, 25-29, 31, 33-40, 58-62, 67-68 and 71 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 25-46 of copending Application No. 11/096,155. (Office Action, pages 12-13).

Applicant requests that the rejection be held in abeyance until the claims of the present application are deemed otherwise allowable. A terminal disclaimer will be submitted in due course if the claims are allowed.

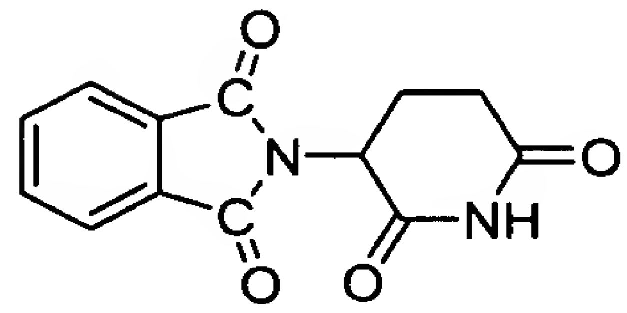
IV. <u>Conclusion</u>

Applicant respectfully requests that the above amendments and remarks be entered in the file of this application. Should the Examiner not agree that all claims are allowable, then a further personal or telephonic interview is respectfully requested to discuss any remaining issues and to accelerate the allowance of the above-identified application. Please charge any required fees to Jones Day Deposit Account No. 50-3013.

Respectfully submitted,

Date: July 12, 2007

By: Yeah-Sil Moon (Reg. No. 52,042)
For Anthony M. Insogna (Reg. No. 35,203)
JONES DAY
222 East 41st Street
New York, NY 10017
Tel. (212) 326-3778



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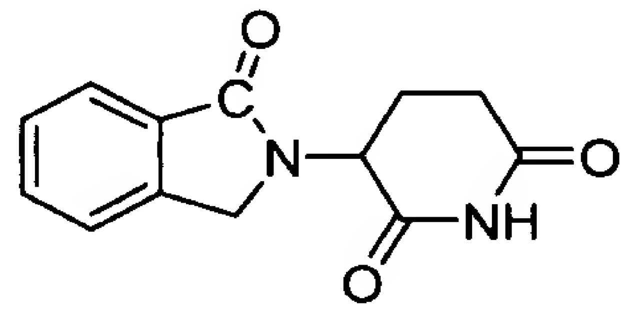

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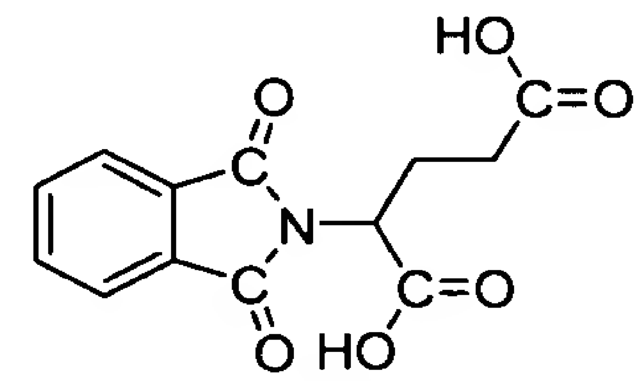

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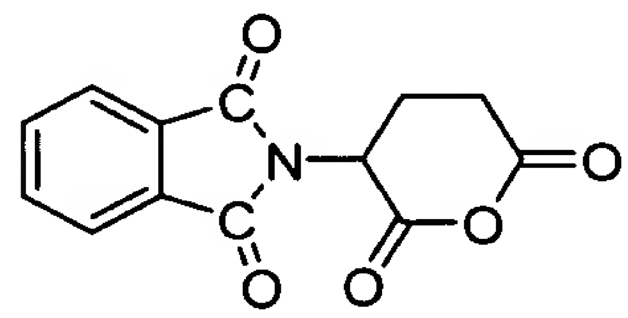

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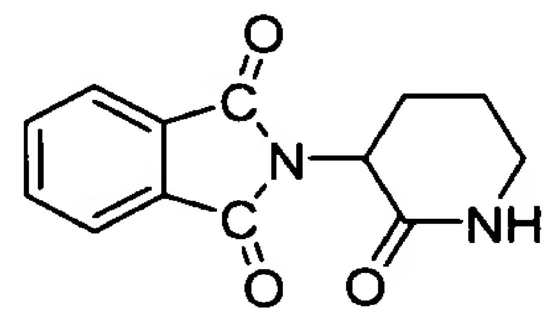

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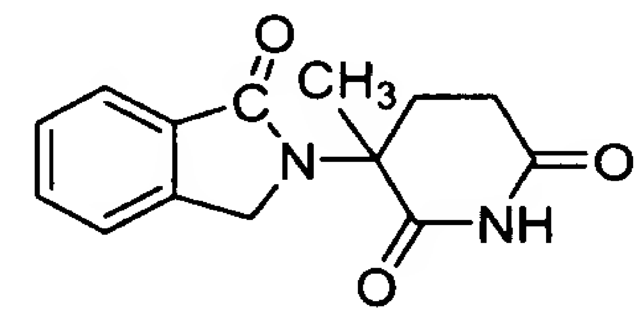

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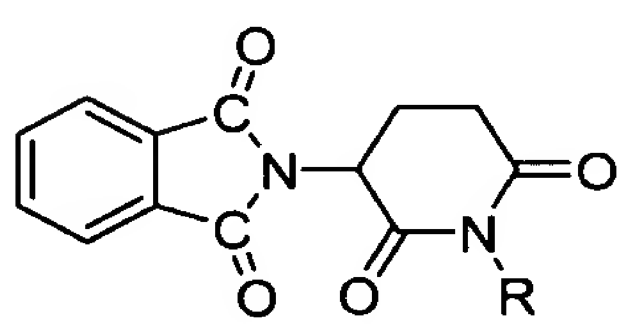

Where R = -H, -OH, or $-CH_3$

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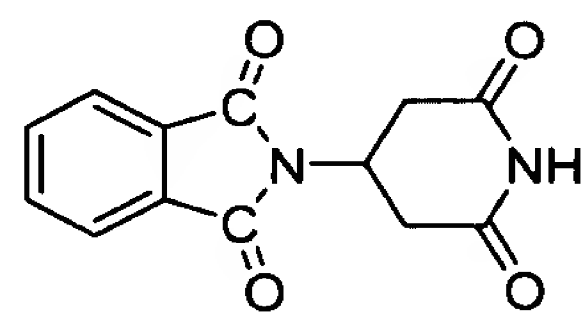

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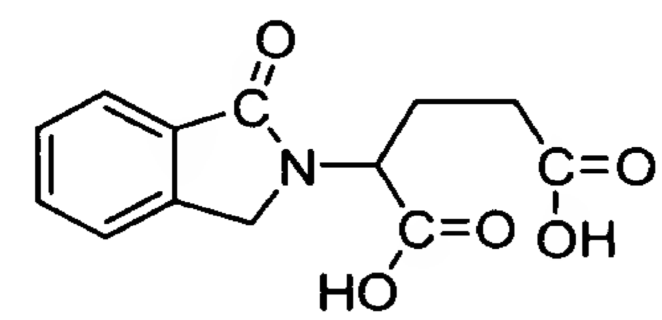

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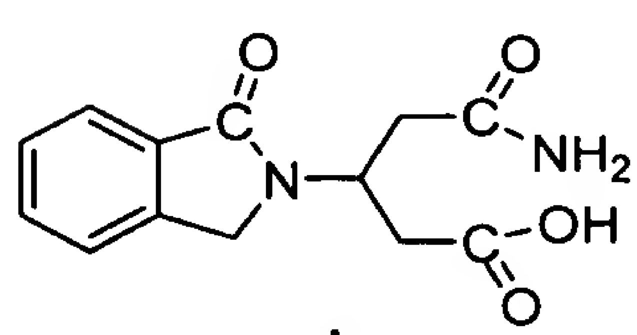

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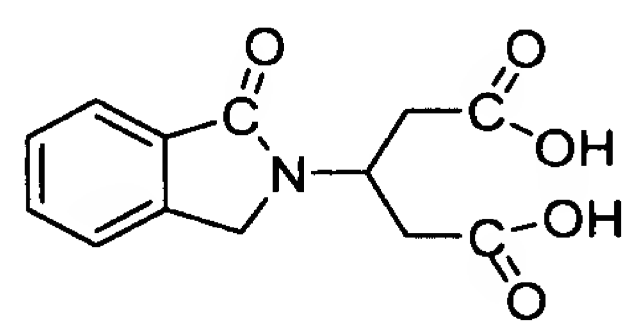

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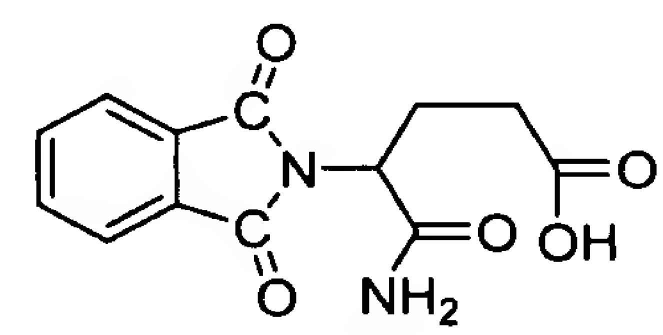

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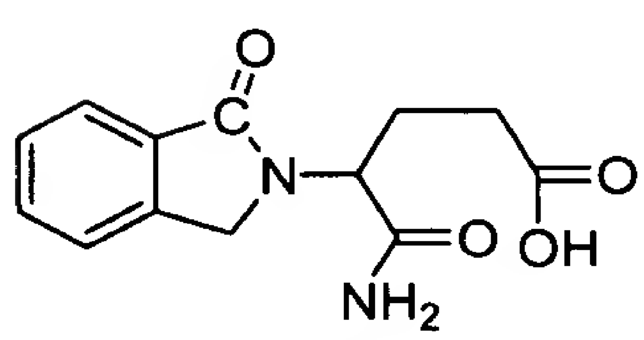

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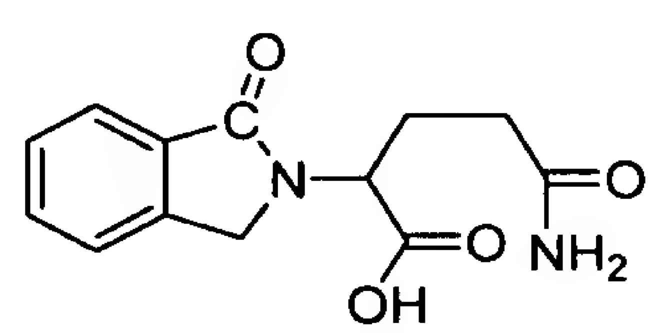

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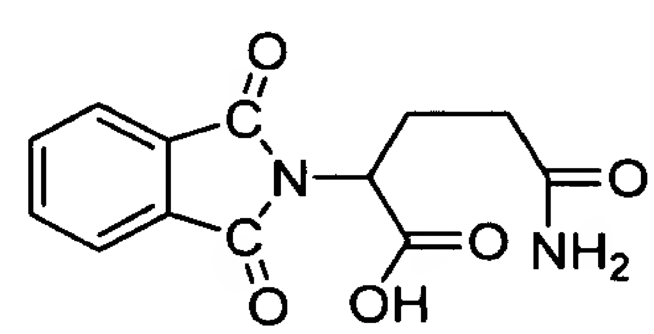

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FIGURE 1

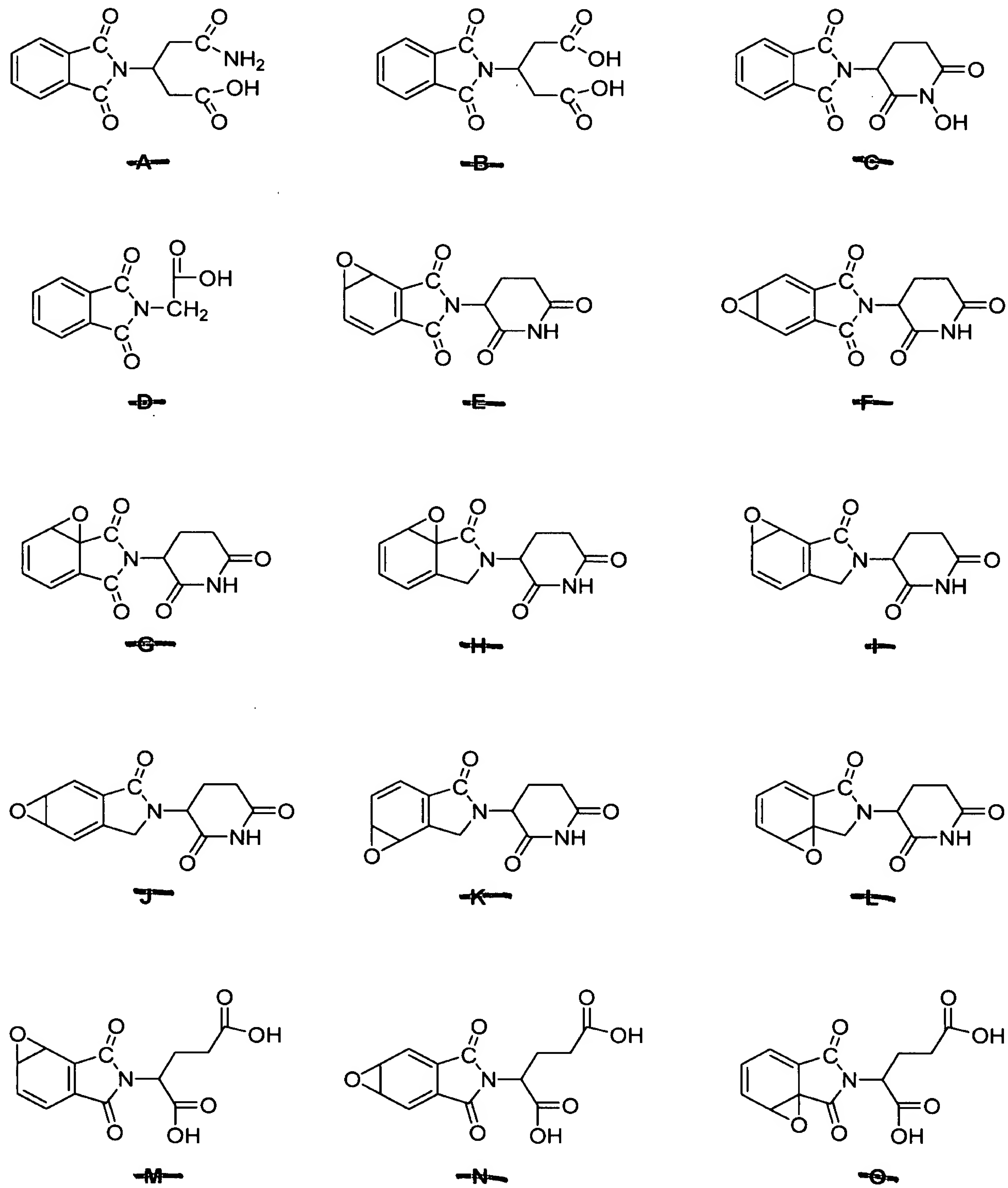


FIGURE 2

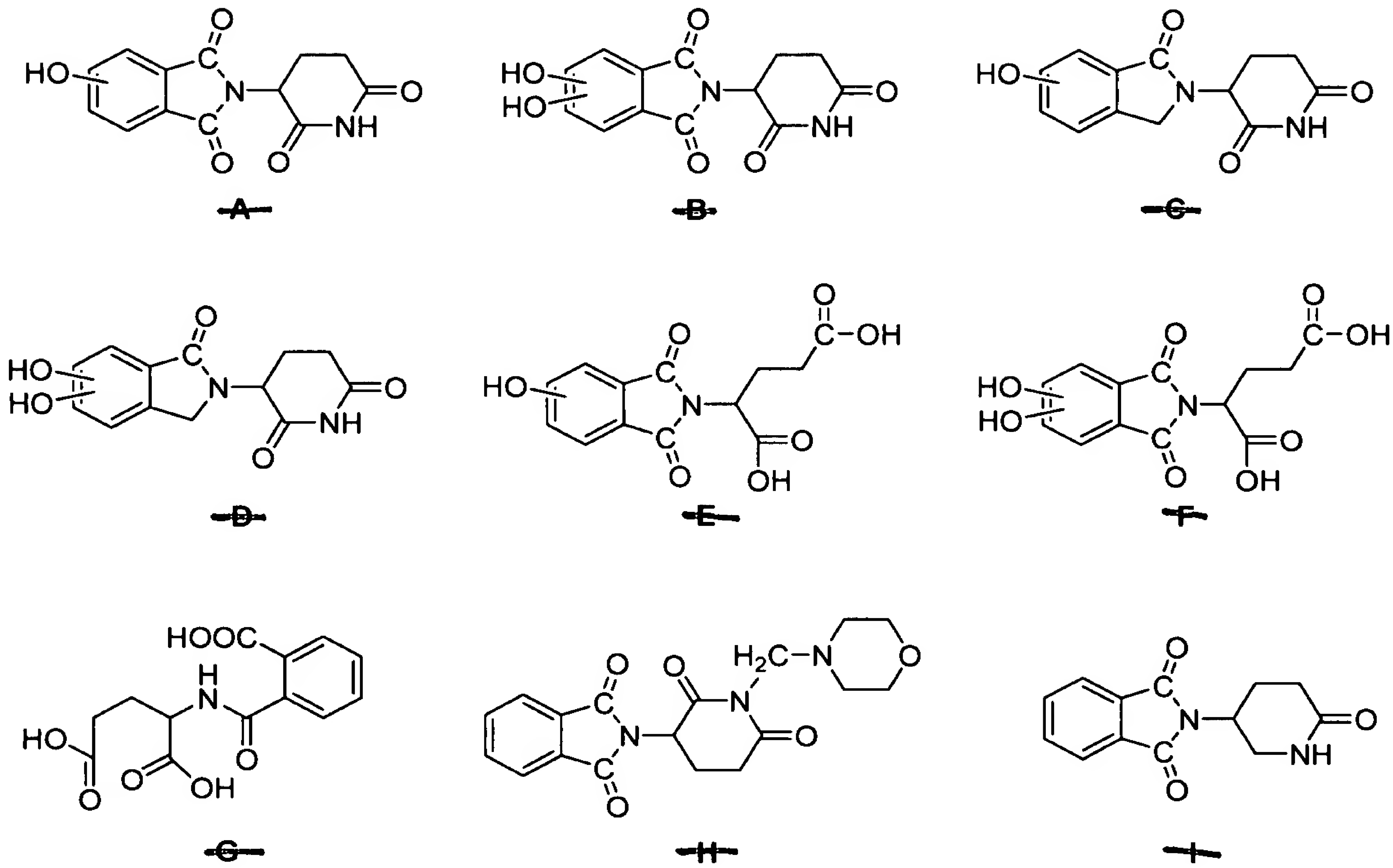


FIGURE 3

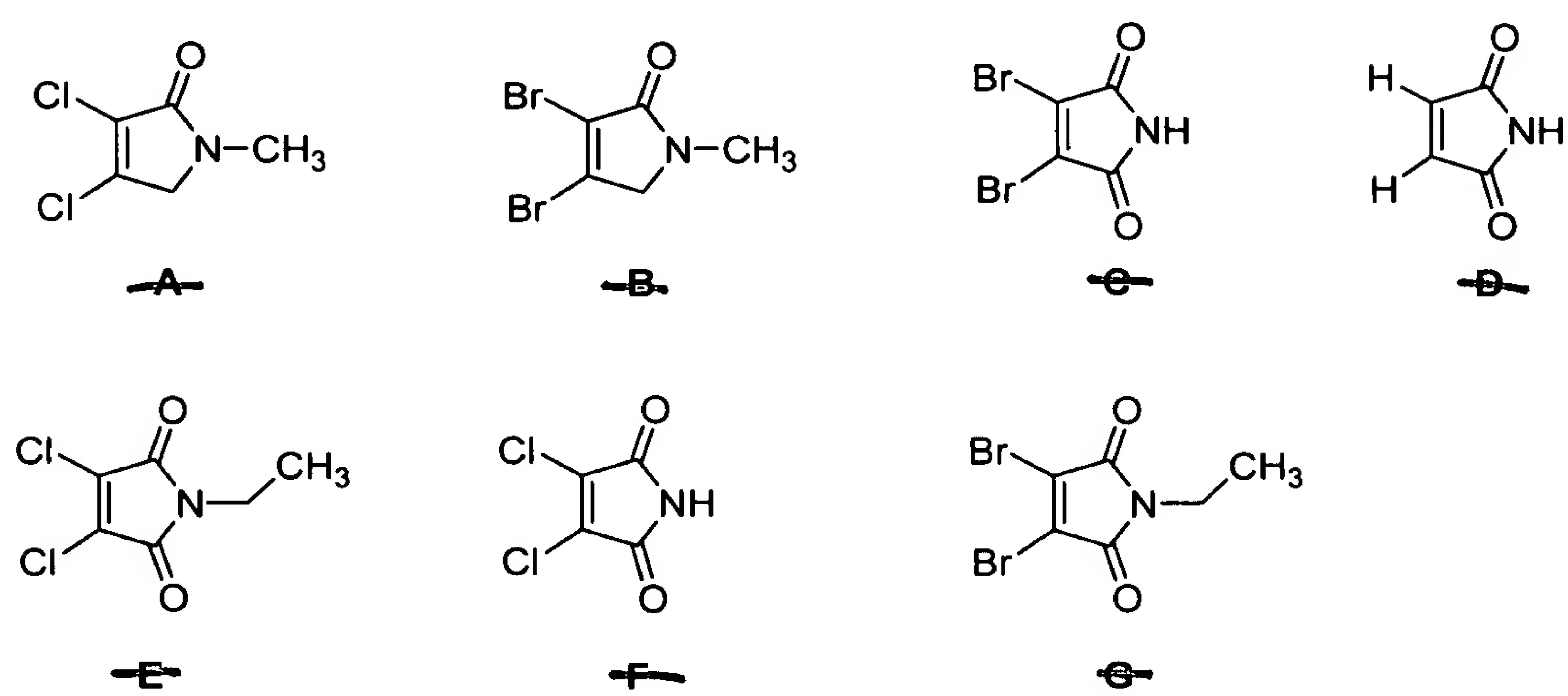
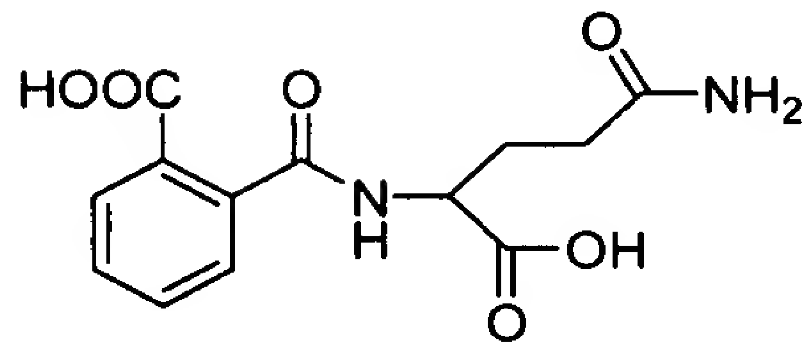


FIGURE 4



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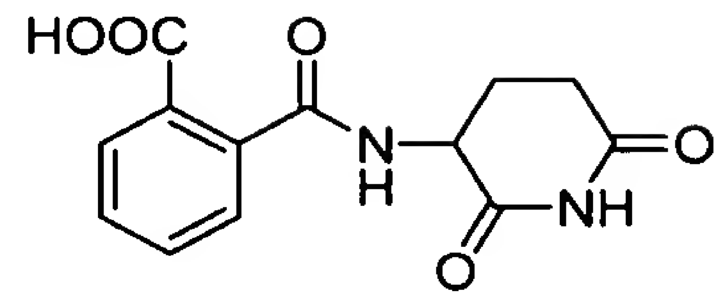

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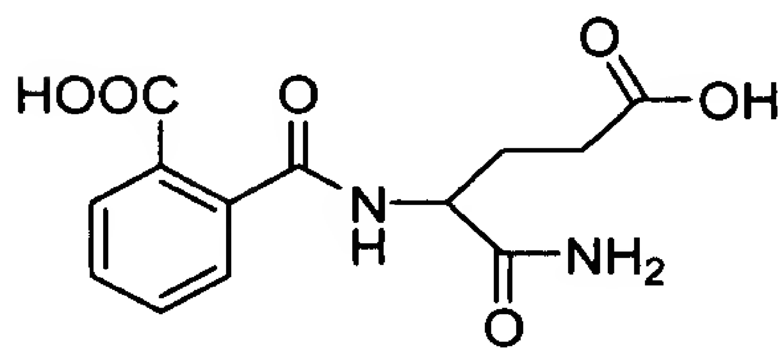

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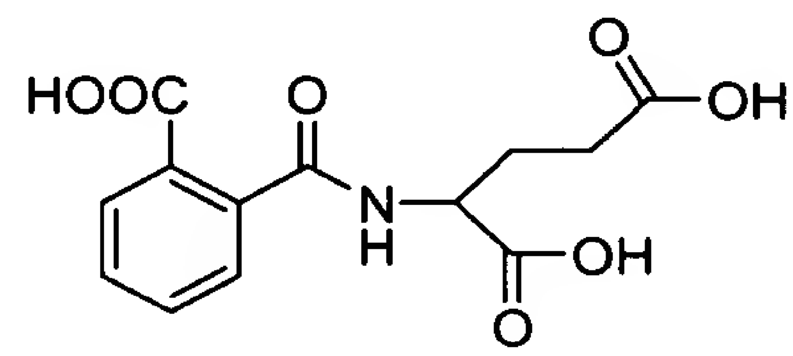

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FIGURE 5

ANTITUMOR ACTIVITY OF THALIDOMIDE IN REFRACTORY MULTIPLE MYELOMA

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ABSTRACT

Background Patients with myeloma who relapse after high-dose chemotherapy have few therapeutic options. Since increased bone marrow vascularity imparts a poor prognosis in myeloma, we evaluated the efficacy of thalidomide, which has antiangiogenic properties, in patients with refractory disease.

Methods Eighty-four previously treated patients with refractory myeloma (76 with a relapse after high-dose chemotherapy) received oral thalidomide as a single agent for a median of 80 days (range, 2 to 465). The starting dose was 200 mg daily, and the dose was increased by 200 mg every two weeks until it reached 800 mg per day. Response was assessed on the basis of a reduction of the myeloma protein in serum or Bence Jones protein in urine that lasted for at least six weeks.

Results The serum or urine levels of paraprotein were reduced by at least 90 percent in eight patients (two had a complete remission), at least 75 percent in six patients, at least 50 percent in seven patients, and at least 25 percent in six patients, for a total rate of response of 32 percent. Reductions in the paraprotein levels were apparent within two months in 78 percent of the patients with a response and were associated with decreased numbers of plasma cells in bone marrow and increased hemoglobin levels. The microvascular density of bone marrow did not change significantly in patients with a response. At least one third of the patients had mild or moderate constipation, weakness or fatigue, or somnolence. More severe adverse effects were infrequent (occurring in less than 10 percent of patients), and hematologic effects were rare. As of the most recent follow-up, 36 patients had died (30 with no response and 6 with a response). After 12 months of follow-up, Kaplan–Meier estimates of the mean (±SE) rates of event-free survival and overall survival for all patients were 22±5 percent and 58±5 percent, respectively.

Conclusions Thalidomide is active against advanced myeloma. It can induce marked and durable responses in some patients with multiple myeloma, including those who relapse after high-dose chemotherapy. (N Engl J Med 1999;341:1565-71.)

MULTIPLE myeloma accounts for approximately 1 percent of all cancers and 10 percent of hematologic cancers. It is incurable with conventional chemotherapy.[1] Melphalan-based high-dose chemotherapy with hematopoietic stem-cell support increases the rate of complete remission and extends event-free and overall survival.[2-4] However, many patients still relapse, and options for salvage therapy are limited.[5,6]

Angiogenesis is important in embryogenesis, wound healing, diabetic retinopathy, and tumor progression.[7,8] The immunomodulatory drug thalidomide can inhibit angiogenesis and induce apoptosis of established neovasculature in experimental models.[9,10] For these reasons, angiogenesis-inhibiting drugs such as thalidomide may be useful for treating cancers that depend on neovascularization.

Prominent bone marrow vascularization occurs in multiple myeloma. It correlates positively with a high plasma-cell–labeling index (a poor prognostic sign) and disease activity and independently confers a poor prognosis.[11-16] Plasma levels of various angiogenic cytokines, such as basic fibroblast growth factor and vascular endothelial growth factor, are elevated in patients with active myeloma.[11-13,16] In 1965, Olson et al. reported slowing of disease progression in one patient who was treated with thalidomide.[17] These considerations led us to administer thalidomide to five patients with end-stage myeloma through a compassionate-use protocol. One patient with a large tumor burden (as indicated by an IgA level of 8.4 g per deciliter, the presence of more than 95 percent plasma cells in bone marrow, and the need for transfusion), who had had no response to two cycles of high-dose chemotherapy followed by multiple salvage therapies, had a nearly complete remission within three months after the initiation of thalidomide therapy. This observation prompted a phase 2 investigation of tha-

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lidomide in patients with advanced and refractory myeloma.

METHODS

Patients and Treatments

Between December 1997 and June 1998, 84 consecutive, eligible patients with previously treated and progressive myeloma began treatment with oral thalidomide as a single agent after providing written informed consent. No patients were excluded on the basis of renal or cardiopulmonary function, whereas patients could be excluded if the results of liver-function tests were more than twice the upper limit of normal levels. All patients were treated at a single center according to a phase 2 protocol approved by the institutional review board and the Food and Drug Administration (FDA).

Thalidomide was supplied in 50-mg capsules by Celgene (Warren, N.J.) and was administered nightly at a dose of 200 mg. The dose was increased by 200 mg every two weeks for six weeks, so that the final dose was 800 mg per day. Data were analyzed as of June 17, 1999, when the duration of treatment ranged from 2 to 465 days (median, 80) and the median follow-up of surviving patients was 13 months.

Table 1 summarizes the characteristics of the patients and details of prior therapy. Seventy-six patients (90 percent) had received at least one cycle of high-dose chemotherapy with autologous hematopoietic stem-cell support, and 58 (69 percent) had received two or more cycles of intensive chemotherapy. The median time from the last course of high-dose chemotherapy to the beginning of treatment with thalidomide was 14 months. A high-risk cytogenetic abnormality (deletion of chromosome 13) was present in 35 patients (42 percent).[20] One patient had received an allograft as a second intervention, with evidence of full donor-type chimerism in normal lymphohematopoietic cells. At the time of enrollment, all patients had progressive disease, with an increase in paraprotein levels of at least 25 percent or at least 50 percent plasma cells in bone marrow. Approximately half the patients had been retreated with dexamethasone or other regimens, but the disease had progressed before thalidomide treatment was begun.

Evaluation

The pretreatment evaluation included complete blood counts, tests of renal and liver function, serum and urine protein electrophoresis, and measurements of serum levels of immunoglobulins, beta$_2$-microglobulin, and C-reactive protein. Bone marrow aspirates were obtained and biopsies were performed to determine the percentage of plasma cells in bone marrow, to identify karyotypic abnormalities (Giemsa-banded cells in metaphase), and to assess the proliferative activity in plasma cells according to the bromodeoxyuridine method to derive the plasma-cell–labeling index.[18] Follow-up studies included a weekly estimation of paraprotein levels — the myeloma protein in serum and Bence Jones protein in urine — for the first two months, followed thereafter by monthly measurements. Whenever possible, bone marrow was examined at the time of the maximal response or when patients with no response left the study.

The microvascularity of bone marrow was studied in a semiquantitative fashion in biopsy samples that were obtained with a trephine and stained with an anti-CD34 monoclonal antibody (prediluted Clone QBEnd/10, Cell Marque, Austin, Tex.). The results were expressed as the number of vessels per high-power field (400×).

Assessment of Response

The primary end point of the study was the finding of a decline in the level of paraprotein in serum or urine of at least 25 percent, 50 percent, 75 percent, or 90 percent on two occasions at least six weeks apart. Among patients with detectable levels of both urine and serum paraprotein, the response was judged on the basis of the component showing the smaller decline. Patients with a reduction of less than 25 percent and those who discontinued treatment before a response could be assessed were considered to have had no response to thalidomide. Thus, the results were evaluated on an intention-to-treat basis. In patients with a response, an increase in serum or urine paraprotein levels by more than 25 percent above the nadir value was considered evidence of relapse. In patients who had a complete remission, evidence of reemergence of the monoclonal protein (determined by immunofixation) on at least two occasions was considered to indicate a relapse. In patients who had a complete remission or a nearly complete remission (≥90 percent reduction in serum or urine paraprotein levels), a bone marrow response was defined as the finding of less than 5 percent plasma cells in the biopsy specimen or aspirate. For the remaining patients with a paraprotein response, the percentage of plasma cells had to decrease by at least 50 percent to qualify as a bone marrow response.

Table 1. Characteristics of the Patients.

Characteristic	No. of Patients (%)
Male sex	61 (73)
Durie–Salmon stage III multiple myeloma	51 (61)
IgG paraprotein	51 (61)
Duration of prior therapy >60 mo	18 (21)
Prior high-dose chemotherapy	76 (90)
Receipt of >1 cycle of high-dose chemotherapy	58 (69)
Interval between last cycle of high-dose chemotherapy and initiation of thalidomide >12 mo	43 (57)
Age >60 yr	32 (38)
Hemoglobin <9 g/dl	19 (23)
Platelet count $<50\times10^3/mm^3$	17 (20)
Serum albumin <3.5 g/dl	22 (26)
Serum creatinine >1.5 mg/dl (133 μmol/liter)	22 (26)
Serum beta$_2$-microglobulin >6 mg/liter	24 (29)
Serum C-reactive protein >3 mg/liter	20 (24)
Serum monoclonal immunoglobulin >1 g/dl	51 (61)
Urine Bence Jones protein >1 g/day	44 (52)
>50% Plasma cells in bone marrow on biopsy	18 (21)
Plasma-cell–labeling index >1%*	13 (15)
Bartl grade II†	19 (23)
Deletion of chromosome 13	35 (42)
Outcome	
Completion of study	19 (23)
Withdrawal from study	
Progression	54 (64)
Intolerance of thalidomide	9 (11)
Death of patient with a response‡	1 (1)
Personal reasons	1 (1)
Final dose of thalidomide	
400 mg/day	72 (86)
600 mg/day	57 (68)
800 mg/day	46 (55)

*The plasma-cell–labeling index represents the percentage of light-chain–restricted plasma cells incorporating bromodeoxyuridine.[18]

†The Bartl grading system distinguishes myeloma cells according to their morphologic maturation.[19] Grade II refers to immature plasma cells of cleaved, asynchronous, or polymorphous appearance.

‡This patient had a response to treatment but died on day 37 of treatment.

Assessment of Adverse Effects

All patients, irrespective of the duration of therapy, were included in the evaluation of adverse effects. All patients received diaries after providing informed consent, and 83 patients (99 per-

cent) reported having adverse effects. A comprehensive checklist of the adverse effects associated with thalidomide therapy was provided by Celgene; it was based on previous experience in treating patients with leprosy and had been reviewed by the FDA. The data were verified by the patients by direct or telephone interviews. Hematologic values and other laboratory-based measures of adverse effects were assessed at least monthly by the data-management office.

Statistical Analysis

The primary end point for this phase 2 study was a diminution in the plasma level of the myeloma protein or the urine level of Bence Jones protein. Other end points included the time to a response, the time to disease progression, event-free survival, overall survival, the microvascularity of bone marrow, and improvements in other laboratory values. Response was treated as a categorical variable. Comparisons of the response according to other categorical variables were assessed with use of the chi-square test or Fisher's exact test, as appropriate. The times to response and disease progression were calculated with the use of the competing-risk methods.[21] The time to response was defined as the interval between the start of therapy and a given response (i.e., a decline in the serum or urine level of paraprotein of at least 25 percent, 50 percent, 75 percent, or 90 percent or a complete remission). Competing risks with respect to the time to response included discontinuation of treatment because of progression or a lack of response, an inability to tolerate thalidomide, or death or personal reasons. The time to progression was calculated only for patients with a paraprotein response and was defined as the time from the start of therapy to disease progression. Competing risks with respect to the time to progression included discontinuation of treatment because of adverse effects or death or for personal reasons. Event-free survival and overall survival were estimated according to the method of Kaplan and Meier.[22] Event-free survival was calculated from the start of therapy to disease progression, removal from the study for any reason, death from any cause, or the last follow-up visit, whichever occurred first. Overall survival was calculated from the start of therapy to death from any cause or the last follow-up visit. Data on patients who had not had an event by the time of the last follow-up were censored at that time with respect to times to response and progression, event-free survival, and overall survival. Survival was compared with use of the log-rank test.[23] Univariate and multivariate (stepwise) logistic-regression methods were used to evaluate the prognostic importance of various characteristics with respect to the likelihood of achieving at least a 25 percent or 50 percent reduction in serum or urine paraprotein levels. Univariate and multivariate (stepwise) proportional-hazards regression analyses were used to evaluate the prognostic importance of various characteristics with respect to event-free survival and overall survival.

Since the microvascular density of bone marrow was used as a measure of the antiangiogenic action of thalidomide, this variable was extensively modeled. To account for the need for multiple measurements of each patient over time and missing data, we used mixed-models repeated-measures analysis of variance to evaluate the microvascular density of bone marrow.[24] The use of compound symmetry and first-order autoregressive covariance structures was compared, and the results were found to be similar according to Akaike's criterion. Therefore, the values obtained with the compound-symmetry models are reported. Measurements of the microvascular density of bone marrow were grouped according to the length of treatment, and values were measured every 50 days for a total of seven times, including the pretreatment value. The natural logarithm of the values for the microvascular density of bone marrow was used in the analysis. Estimates for patients with no response and patients with a complete or nearly complete response (≥90 percent reduction in serum or urine paraprotein levels) were used to predict the response in terms of the microvascular density of bone marrow over time.

Improvements in important clinical measures were evaluated on the basis of the percent change from base line to the time of the maximal response or, for those without a response, the time at which treatment was discontinued. Spearman correlations were used to assess whether the changes within response groups were significant. For variables with no significant correlations, the signed-rank test was used to test the hypothesis within response groups that the change was significantly different from zero. All statistical tests were two-sided.

RESULTS

Decline in Paraprotein Levels

Timely escalations in the daily dose of thalidomide to 400 mg, 600 mg, and 800 mg were possible in 83 percent, 62 percent, and 47 percent of the patients, respectively; the proportions of patients who eventually reached these levels were 86 percent, 68 percent, and 55 percent, respectively (Table 1). In 27 patients (32 percent), the serum or urine paraprotein level declined by at least 25 percent, including 7 (8 percent) with a decline of at least 50 percent, 6 (7 percent) with a decline of at least 75 percent, and 6 (7 percent) with a decline of at least 90 percent; 2 patients had a complete remission (Table 2). The median interval between the start of treatment and a decrease in the paraprotein level of at least 25 percent was 29 days (range, 4 days to 6 months) (Fig. 1). Seventy-eight percent of the responses of this magnitude were apparent within two months; they were observed within four months in all but two patients with a response. More marked reductions in paraprotein, by at least 50 percent and 75 percent, occurred after a median of two and three months of therapy, respectively.

A low plasma-cell–labeling index (assessed as a continuous variable) was the only statistically significant variable associated with a response among both the group with at least a 25 percent decrease in paraprotein levels ($P=0.01$) and the group with at least a 50 percent decrease ($P=0.01$). Using the median plasma-cell–labeling index of 0.2 percent as a cutoff value, we found that 46 percent of patients with values below the median had a reduction in paraprotein levels of at least 25 percent, as compared with 9 percent of patients with higher values ($P<0.05$). On univariate analysis, deletion of chromosome 13 was predictive of an unfavorable response, but not on multivariate analysis.

Bone Marrow Response

Bone marrow samples were obtained after one to nine months of therapy (median, three) in 48 patients. A paraprotein response was associated with a bone marrow response in 81 percent of the patients who could be evaluated (Table 2). In seven of the eight patients with at least a 90 percent reduction in paraprotein levels, the concurrently examined bone marrow specimens contained less than 5 percent plasma cells. A decline in the percentage of plasma cells in bone marrow by at least 50 percent occurred in only 4 of 27 patients with no paraprotein response (15 percent) who had follow-up bone marrow examinations.

Table 2. Paraprotein Response and Bone Marrow Response.

Paraprotein Response	No. of Patients (% of total)	Assessment of Bone Marrow Response		Current Status	
		Total No.*	No. with Response (%)†	No. with Relapse (%)	No. Who Died (%)
Complete remission	2 (2)	2	2 (100)	0	0
≥90% decrease in paraprotein	6 (7)	6	5 (83)	2	2
≥75% decrease in paraprotein	6 (7)	5	3 (60)	3	1
≥50% decrease in paraprotein	7 (8)	4	4 (100)	3	0
≥25% decrease in paraprotein	6 (7)	4	3 (75)	4	3
Total	27 (32)	21	17 (81)	12 (44)	6 (22)
No response	57 (68)	27	4 (15)	—	30 (53)

*The response could not be evaluated in 6 of the patients with a paraprotein response and in 30 of the patients with no paraprotein response.

†A bone marrow response was defined as the presence of less than 5 percent plasma cells in bone marrow in patients who had a complete paraprotein response or at least a 90 percent reduction in paraprotein levels and as a reduction in plasma cells of at least 50 percent in patients with all other types of paraprotein responses.

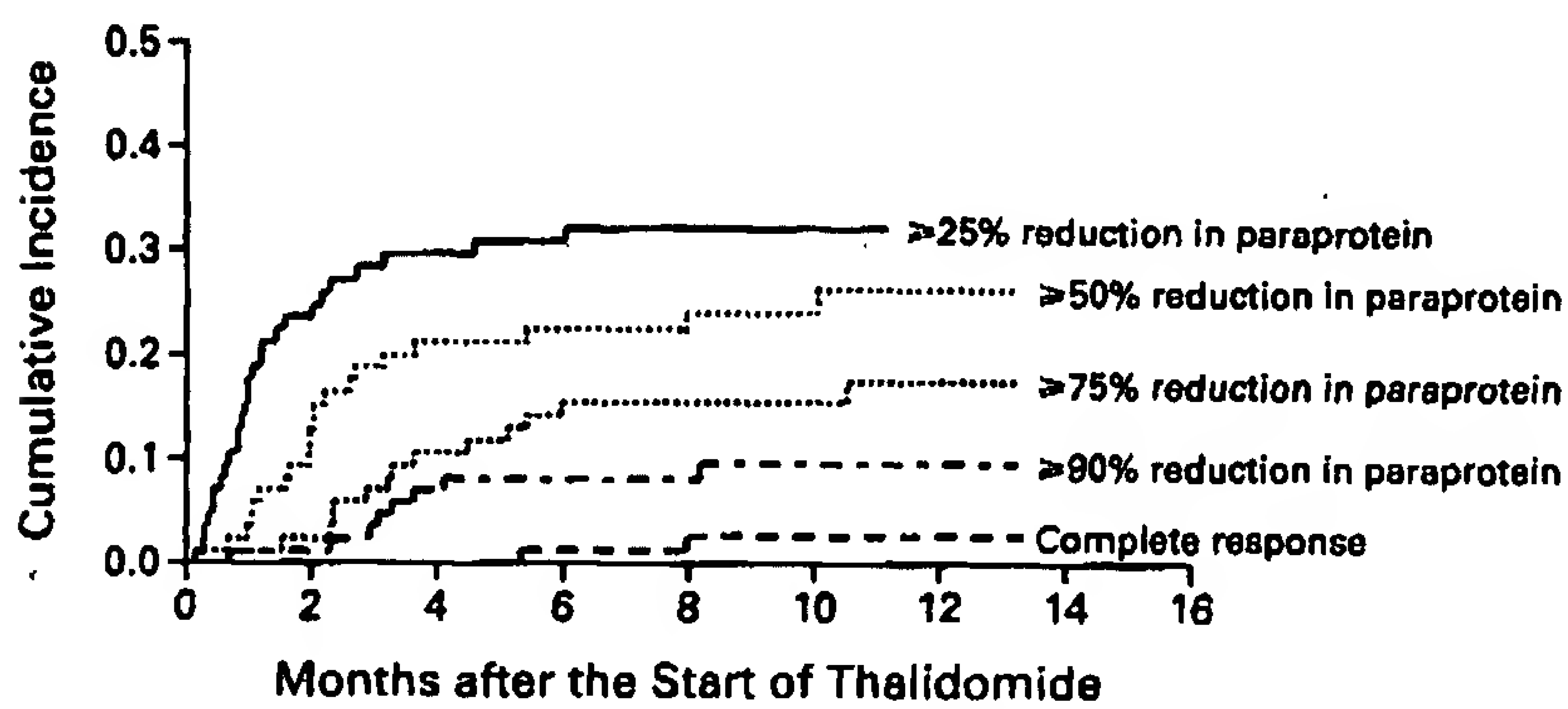


Figure 1. Times to Various Paraprotein Responses.

Among patients with a response, the median times to a reduction in the serum or urine paraprotein level of at least 25 percent, 50 percent, 75 percent, and 90 percent were one, two, four, and four months, respectively. Seventy-eight percent of the responses at the lowest level (≥25 percent reduction) were apparent within two months after the initiation of treatment.

Microvascular Density of Bone Marrow

The microvascular density of bone marrow was scheduled to be assessed every 50 days for a total of seven measurements, including the pretreatment value. At least one measurement of the microvascular density of bone marrow was made in 74 patients (88 percent); two or more measurements were made in 37 patients (44 percent). In all, measurements were made in 69 patients before treatment and (in 50-day increments) in 17 at time 2, in 22 at time 3, in 11 at time 4, in 12 at time 5, in 4 at time 6, and in 3 at time 7. The microvascular density of bone marrow and the percentage of plasma cells in bone marrow correlated significantly at all times except the last ($r>0.5$, $P\leq0.01$). Although the microvascular density of bone marrow decreased markedly in some patients with a complete or nearly complete remission, estimates of the slope were not significantly different from zero among those with a response ($P=0.39$) and those without a response ($P=0.22$).

Other Changes

The percent changes from base line to the time of the maximal response among patients with a response and the time of the last follow-up visit among those without a response were assessed for beta$_2$-microglobulin, C-reactive protein, lactic dehydrogenase, creatinine, albumin, and hemoglobin levels and the platelet count. Hemoglobin levels increased only in patients with a response (median increase, 11 percent; $P<0.001$ for the comparison with base-line values). Serum levels of beta$_2$-microglobulin rose (median in-

crease, 43 percent; P<0.001) and serum albumin levels fell (median decrease, 4 percent; P<0.001) significantly in patients with no response. Serum creatinine levels did not change significantly in patients with a response, and they increased by a median of 13 percent in those without a response (P<0.001).

Adverse Effects

Side effects reported by at least 10 percent of patients at most dose levels are listed in Table 3. Most adverse effects were mild or moderate (grade 1 or 2 according to the system of classification of the World Health Organization). Constipation, weakness or fatigue, and somnolence occurred in one third or more of the patients. Reports of grade 3 or 4 adverse effects were infrequent (less than 10 percent in all cases). One quarter of the patients had no appreciable side effects at the 200-mg dose, whereas virtually all patients had adverse effects of grade 1 or 2 at higher doses. Fewer than 5 percent of patients had grade 1 or 2 leukopenia at any dose, and grade 3 or 4 thrombocytopenia or anemia occurred in only three patients. In most of the patients who had no response, pretreatment anemia or thrombocytopenia did not worsen, whereas significant increases in the hemoglobin levels occurred in patients with a response. Nine patients could not tolerate thalidomide (four with a response and five with no response) and discontinued treatment after a median of 36 days (range, 10 to 241). In eight patients, an increase in serum creatinine levels of more than 50 percent was related to progressive disease, with increasing Bence Jones proteinuria. One of the patients with a response died suddenly on day 37 of treatment. The death was thought to be related to sepsis, although a possible contribution of thalidomide could not be ruled out.

Time to Progression, Event-free Survival, and Overall Survival

Of the 27 patients with a decrease in paraprotein levels of at least 25 percent, 12 had a recurrence of the disease. After a median follow-up of 14.5 months (range, 12 to 16), the median time to progression had not been reached. The disease in a mean (±SE) of 44±10 percent of patients was judged to have progressed at 12 months. The median event-free survival for all 84 patients was three months (Fig. 2). After 12 months of follow-up, 22±5 percent of the 84 patients remained event-free and 58±5 percent were alive. Nineteen patients were still receiving thalidomide 4 to 15 months after starting the treatment (median, 13), including 15 patients with a response and 4 with no response who had had some improvement in various disease indicators but who had not had a decrease in paraprotein levels of at least 25 percent. Multivariate analysis indicated that increases in lactic dehydrogenase levels (P=0.001), the plasma-cell–labeling index (P=0.006), and C-reactive protein levels (P=0.007) were all predictive of a brief period of event-free survival, whereas low albumin levels (P<0.001), the deletion of chromosome 13 (P=0.004), and high numbers of plasma cells in bone marrow (P=0.05) were associated with a relatively short overall survival.

Thalidomide was discontinued after a median of 52 days (range, 2 to 286) because of a lack of response in 53 patients (4 patients continued to receive the drug without a response) and because of relapse in 12 patients who had had a response. One patient who had a decrease in the paraprotein level of at least 25 percent and who had not previously received high-dose therapy subsequently underwent autologous stem-cell transplantation at his own request. As of June 17, 1999, 36 patients had died, including 30 patients without a response who died of progressive disease or complications of subsequent salvage therapy, as well as 6 patients with a response who subsequently relapsed and died of progressive disease (3) or toxicity from salvage therapy (3).

Table 3. Incidence of Grade 1 or 2 Adverse Effects.*

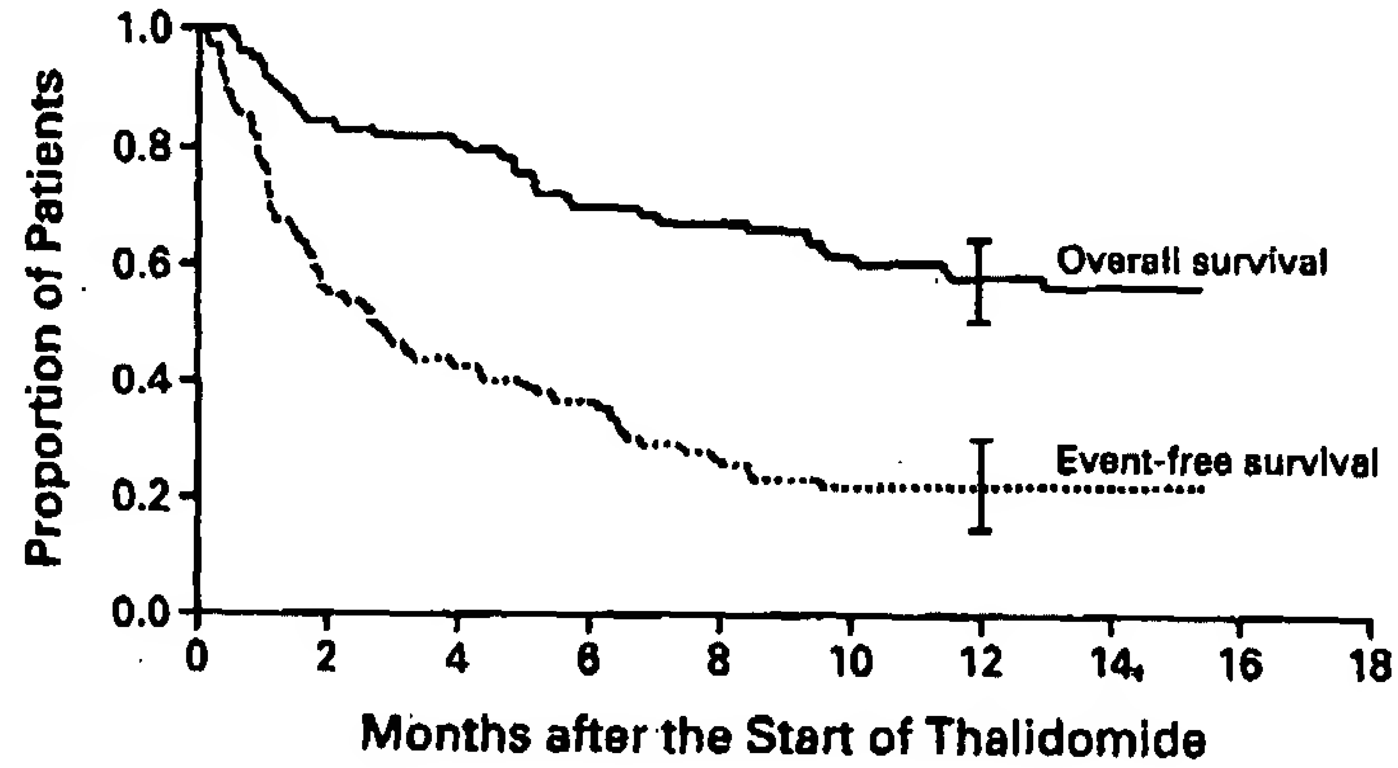
Adverse Effect	Dose of Thalidomide: 200 mg/day (N=83)	400 mg/day (N=72)	600 mg/day (N=57)	800 mg/day (N=46)
	percentage of patients†			
Constipation	35	44	44	59
Weakness or fatigue	29	31	39	48
Somnolence	34	43	40	43
Tingling or numbness	12	14	19	28
Dizziness	17	25	23	28
Rash	16	18	21	26
Mood changes or depression	16	24	23	22
Incoordination	16	17	14	22
Tremors	10	13	19	22
Edema	6	10	12	22
Nausea	12	15	23	11
Headache	12	10	14	11

*The classification system of the World Health Organization was used. Grade 1 effects are mild, and grade 2 are moderate.

†Values are the percentages of patients at each dose level.

DISCUSSION

We found that thalidomide had substantial antitumor activity in patients with advanced myeloma. Ten percent of patients had complete or nearly complete remission, and 32 percent had a reduction in serum or urine paraprotein levels of at least 25 percent. In most patients, the decline in paraprotein levels was accompanied by a reduction in the percentage of plasma cells in bone marrow and an increase in hemoglobin levels, both of which are consistent with the



No. at Risk								
Overall survival	84	78	69	64	58	56	51	34
Event-free survival	84	65	39	32	24	19	18	11

Figure 2. Kaplan–Meier Estimates of Overall Survival and Event-free Survival.
Event-free survival was calculated from the start of thalidomide therapy to progression, removal from the study for any reason, death from any cause, or the last follow-up visit, whichever occurred first. I bars indicate standard errors at 12 months.

presence of a true antitumor effect. Although not examined quantitatively, bone pain decreased markedly in patients with a response. We did not evaluate lytic bone lesions, which seldom heal, even in patients with a sustained complete remission.

Thalidomide has a number of properties that could explain its activity in myeloma; it can alter the expression of adhesion molecules,[25] suppress the production of tumor necrosis factor α,[26] increase the production of interleukin-10,[27] and enhance cell-mediated immunity by directly stimulating cytotoxic T cells.[28] Its interactions with type 1 and type 2 helper T cells produce complex effects on the levels of cytokines such as interleukin-4, interleukin-5, and interferon-γ.[29] Thalidomide also increases the total number of lymphocytes as well as CD8+ and CD4+ T-cell counts, along with substantially increasing mean plasma levels of soluble interleukin-2 receptor.[29]

Thalidomide has been shown to inhibit angiogenesis induced by fibroblast growth factor and vascular endothelial growth factor in a rabbit-cornea micropocket assay[9] and a murine model of corneal vascularization.[10] It has also been shown to cause apoptosis of established tumor-associated angiogenesis in experimental models.[10] The bone marrow of patients with hematologic cancers shows extensive vascularity,[12,13] which has prognostic implications in myeloma.[14] The apparent lack of a consistent decrease in the microvascular density of bone marrow in patients in whom thalidomide had a marked antitumor effect requires further study. The persistence of extensive vascularization in some patients with a response is consistent with the finding of persistent neovascularity in patients with multiple myeloma who had a response to high-dose chemotherapy.[15] The production of angiogenic cytokines such as fibroblast growth factor and vascular endothelial growth factor by undetectable residual myeloma cells may sustain the increased microvascular density of bone marrow in patients considered to be in remission on the basis of bone marrow findings. The persistence of extensive vascularization in patients with a response makes it seem likely that the antimyeloma action of thalidomide depends on more than one of the actions of the drug outlined above. The mouse model of severe combined immunodeficiency, which can be used for the in vivo growth of primary human myeloma cells, is ideally suited to study the mechanisms by which thalidomide induces responses in myeloma.[30]

The antitumor properties of thalidomide are being evaluated in various malignant diseases,[31-35] although only limited efficacy data are available so far. Prolonged responses to thalidomide in some patients with advanced refractory disease suggest that the mechanism of action of thalidomide is distinctly different from that of the other agents active against myeloma. The absence of myelosuppressive and other important adverse effects suggests that thalidomide could be an ideal agent for use in combination with chemotherapy. Indeed, a complete remission has been achieved with such an approach in several patients with myeloma who had no response to treatment with either regimen alone.[36] This approach has also been shown to have greater antitumor activity than chemotherapy alone in a murine model of breast cancer.[37]

In our study, most patients had adverse effects, but the majority of these reactions were mild or moderate. Reducing the dose of thalidomide alleviated the effects in most cases, and only nine patients discontinued therapy altogether. The gradual reduction in

drowsiness and fatigue in some patients with continued treatment at the same dose (data not shown) suggests the occurrence of tachyphylaxis.

We conclude that thalidomide is active against multiple myeloma, even in patients who relapsed after repeated cycles of high-dose chemotherapy. Larger studies of thalidomide, its analogues, and other inhibitors of angiogenesis are therefore warranted in patients with myeloma and other cancers. We are currently evaluating thalidomide in combination with chemotherapy for patients with newly diagnosed multiple myeloma.

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Dr. Zeldis is an employee of Celgene Corporation, and Drs. Mehta and Singhal own stock in Celgene, which manufactures thalidomide.

We are indebted to Beth Wolmer for her persistence in recommending the clinical evaluation of thalidomide in the treatment of multiple myeloma; to the members of the myeloma data-management team for their dedication; and to Caran Swanson for her excellent secretarial assistance. This article is dedicated to the memory of Ira Wolmer, M.D.

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